PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

BOULT WADE TENNANT
Verulam Gardens
70 Gray's Inn Road
London WC1X 8BT
GRANDE BRETAGNE

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

Date of mailing *(day/month/year)*	05. 06. 2001
Applicant's or agent's file reference SCB/51598001	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB99/04182	10/12/1999	10/12/1998

Applicant
THE UNIVERSITY OF NOTTINGHAM et al

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/

European Patent Office - P.B. 5818 Patentlaan 2
NL-2280 HV Rijswijk - Pays Bas
Tel. +31 70 340 - 2040 Tx: 31 651 epo nl
Fax: +31 70 340 - 3016

Authorized officer

Cardenas, C

Tel.+31 70 340-3370

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
SCB/51598001		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB99/04182	10/12/1998	10/12/1998
International Patent Classification (IPC) or national classification and IPC G01N33/574		
Applicant THE UNIVERSITY OF NOTTINGHAM et al		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☒ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
10/07/2000	05. 06. 2001
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized officer Van Bohemen, C Telephone No. +31 70 340 2199

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB99/04182

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-20 as originally filed

Claims, No.:

1-51 as originally filed

Drawings, sheets:

1/8-8/8 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB99/04182

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-51
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-51
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-51
	No:	Claims	

2. Citations and explanations
see separate sheet

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

VII. Certain defects in the international application

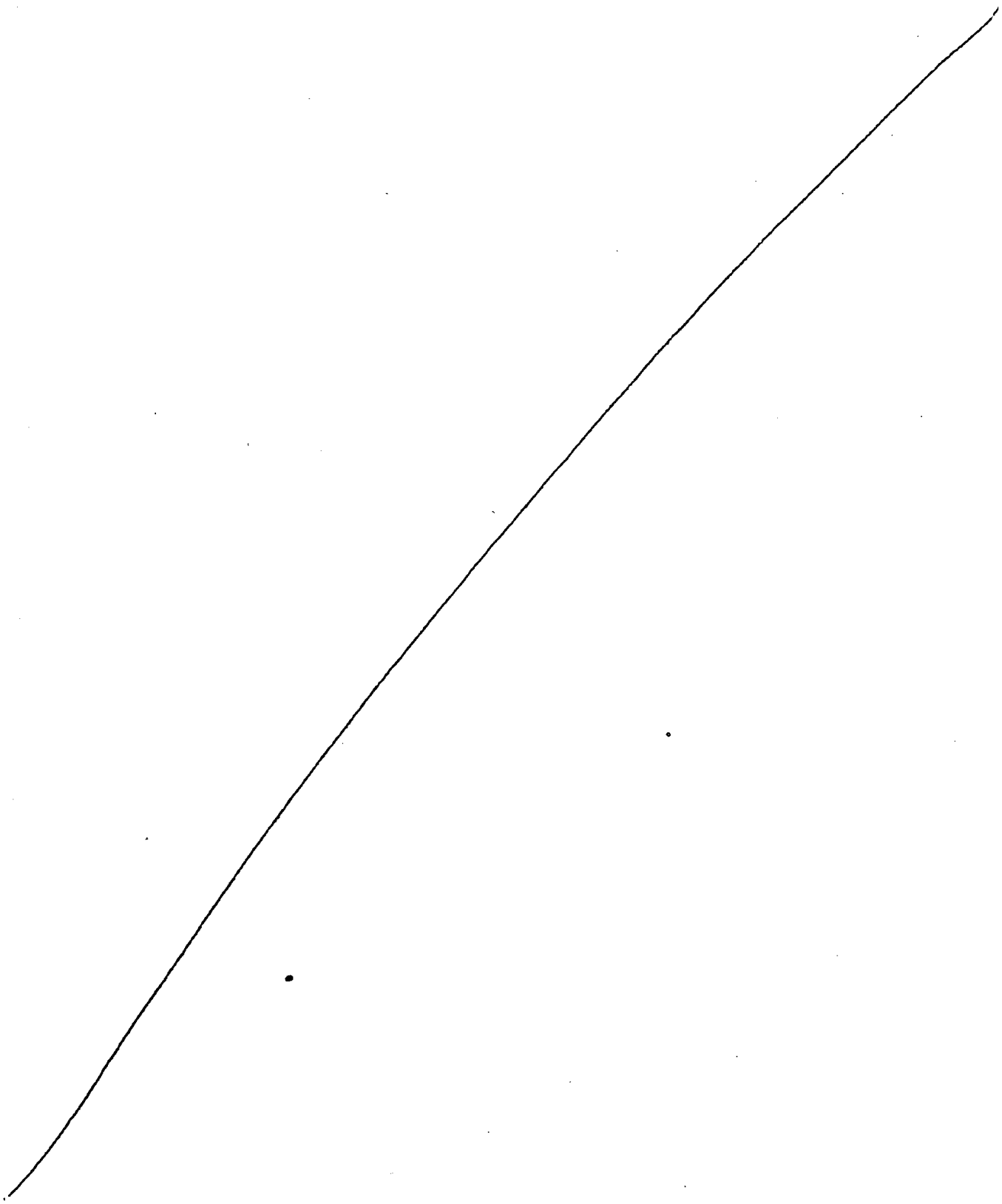
The following defects in the form or contents of the international application have been noted:
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB99/04182



INTERNATIONAL PRELIMINARY EXAMINATION REPORT - SEPARATE SHEET

International application No. PCT/GB99/04182

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following document, which has been cited in the international search report:

D1: E. Petrakou et al. (1997). Preliminary studies on the binding of human autoantibodies to the MUC1 antigen. International Journal of Oncology, (1997) Vol. 11, No. SUPPL., pp. 902.

The present application (PA) appears to meet the requirements of Article 33(1) and (3) PCT, because the subject-matter of claims 1 -51 appears to be new, inventive and industrially applicable in the sense of Article 33(1) - (3) and Rule 64 PCT.

Document D1 is identified as the closest prior art. D1 discloses an *in vitro* method for detecting anti-MUC1 autoantibodies in a sample of body fluid (e.g. a serum sample) using MUC1 antigen preparations (cf D1, abstract). The MUC1 antigen preparation of D1 may be normal urinary MUC1, protein associated MUC1, cancer-associated MUC1 or synthetic MUC1. The disclosure of the PA is based on the use of autoantibodies to detect the presence of a cancer-associated antigens (e.g. MUC1) in samples of body fluids; this is the immunological opposite of the method of D1. To this end, the PA surprisingly discloses that autoantibodies having specificity for cancer-associated antigens can be isolated from serum and subsequently used to detect the presence of cancer-associated antigen in a sample of body fluid. Furthermore, said autoantibodies show very low cross-reactivity with wild-type forms of cancer-associated antigens and possess far higher sensitivities than the antibodies currently used in tests to detect cancer-associated antigens.

The artisan could find no indication for the above in the closest prior art D1 or any other prior art disclosed in the international search report or the description of D1.

Benefit of the above is the ability to detect cancer-associated marker proteins from early stages of disease.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT - SEPARATE SHEET

International application No. PCT/GB99/04182

In summary, as presently formulated novelty, inventivity and industrial applicability of the methods, reagents, cell lines and kits of claims 1-51 should apparently be recognized (cf. 33(1)-(3) and Rule 64 PCT).

Re Item VI
Certain documents cited

Depending on whether or not the present application validly claims a priority date earlier than the international filing date, the following document, which was cited in the international search report, might be considered in the course of further procedures.

WO-A-9958978 (UNIVERSITY OF NOTTINGHAM)

Re Item VII
Certain defects in the international application

Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in the document D1 is not mentioned in the description, nor is this document identified therein.

Re Item VIII
Certain observations on the international application

1. Claims 2 and 48-51 are not clear ex art. 6 PCT, because the quantitative term "substantially" is not clear; the artisan would not automatically understand which degree or quantity is intended.

2. Claims 47-51 are not clear ex art. 6 PCT, because the phrase "as described herein with reference to the accompanying examples" is not clear; the artisan would not automatically understand which method (cf. claim 48), kit (cf. claim 49), reagent (cf. claim 50) or cell population (cf. claim 51) is intended.

3. Regarding to use of separate independent claims relating to the same subject-matter the following.

a. Although claims 1 and 48 have been drafted as separate independent claims, they appear to relate effectively to the same subject-matter. It would appear that conciseness and clarity ex art. 6 PCT could be enhanced by defining the relevant subject-matter in terms of a single independent claim, followed by dependent claims covering features which are merely optional.

b. The above also applies to claims 20 and 50.

c. The above also applies to claims 30 and 51.

d. The above also applies to claims 40 and 49.

PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only

International Application No.

International Filing Date 09/857739

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference *(if desired) (12 characters maximum)* SCB/51598001

Box No. I TITLE OF INVENTION

CANCER DETECTION METHOD AND REAGENTS

Box No. II APPLICANT

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

THE UNIVERSITY OF NOTTINGHAM
UNIVERSITY PARK
NOTTINGHAM
NG7 2RD
UNITED KINGDOM

☐ This person is also inventor.

Telephone No.

Facsimile No.

Teleprinter No.

State *(that is, country)* of nationality: GB

State *(that is, country)* of residence: GB

This person is applicant for the purposes of: ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

ROBERTSON: JOHN RUSSELL
16 BARRATT LANE
ATTENBOROUGH
NOTTINGHAM
NG9 6AF
UNITED KINGDOM

This person is:

☐ applicant only

☒ applicant and inventor

☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is,country)* of nationality: GB

State *(that is, country)* of residence: GB

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

☒ Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: ☒ agent ☐ common representative

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

BOULT WADE TENNANT
27 FURNIVAL STREET
LONDON EC4A 1PQ
UNITED KINGDOM

Telephone No. +44 (0)20 7430 7500

Facsimile No. +44 (0)20 7831 1768

Teleprinter No.

☐ **Adress for correspondence:** Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Continuation of Box No. III **FURTHER APPLICANTS AND/OR (FURTHER) INVENTORS**

If none of the following sub-boxes is used, this sheet should not be included in the request.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

GRAVES: CATHERINE ROSAMUND LOUISE
6 LINDY CLOSE
KINOULTON
NOTTINGHAM
NG7 3RD
UNITED KINGDOM

This person is:
- ☐ applicant only
- ☒ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: GB

State *(that is, country)* of residence: GB

This person is applicant for the purposes of:
- ☐ all designated States
- ☐ all designated States except the United States of America
- ☒ the United States of America only
- ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

PRICE: MICHAEL RAWLING
351 DERBY ROAD
NOTTINGHAM
UNITED KINGDOM

This person is:
- ☐ applicant only
- ☒ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: GB

State *(that is, country)* of residence: GB

This person is applicant for the purposes of:
- ☐ all designated States
- ☐ all designated States except the United States of America
- ☒ the United States of America only
- ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:
- ☐ applicant only
- ☐ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of:
- ☐ all designated States
- ☐ all designated States except the United States of America
- ☐ the United States of America only
- ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:
- ☐ applicant only
- ☐ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of:
- ☐ all designated States
- ☐ all designated States except the United States of America
- ☐ the United States of America only
- ☐ the States indicated in the Supplemental Box

☐ Further applicants and/or (further) inventors are indicated on another continuation sheet.

Box No.V DESIGNATION OF STATES

The following designations are hereby made under Rule 4.9(a) *(mark the applicable check-boxes; at least one must be marked)*:

Regional Patent

- [X] **AP** **ARIPO Patent:** **GH** Ghana, **GM** Gambia, **KE** Kenya, **LS** Lesotho, **MW** Malawi, **SD** Sudan, **SL** Sierra Leone, **SZ** Swaziland, **UG** Uganda, **ZW** Zimbabwe, and any other State which is a Contracting State of the Harare Protocol and of the PCT
- [X] **EA** **Eurasian Patent:** **AM** Armenia, **AZ** Azerbaijan, **BY** Belarus, **KG** Kyrgyzstan, **KZ** Kazakhstan, **MD** Republic of Moldova, **RU** Russian Federation, **TJ** Tajikistan, **TM** Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT
- [X] **EP** **European Patent:** **AT** Austria, **BE** Belgium, **CH and LI** Switzerland and Liechtenstein, **CY** Cyprus, **DE** Germany, **DK** Denmark, **ES** Spain, **FI** Finland, **FR** France, **GB** United Kingdom, **GR** Greece, **IE** Ireland, **IT** Italy, **LU** Luxembourg, **MC** Monaco, **NL** Netherlands, **PT** Portugal, **SE** Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT
- [X] **OA** **OAPI Patent:** **BF** Burkina Faso, **BJ** Benin, **CF** Central African Republic, **CG** Congo, **CI** Côte d'Ivoire, **CM** Cameroon, **GA** Gabon, **GN** Guinea, **GW** Guinea-Bissau, **ML** Mali, **MR** Mauritania, **NE** Niger, **SN** Senegal, **TD** Chad, **TG** Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT *(if other kind of protection or treatment desired, specify on dotted line)* . . . . . .

National Patent *(if other kind of protection or treatment desired, specify on dotted line)*:

- [X] **AE** United Arab Emirates
- [X] **AL** Albania . . . . .
- [X] **AM** Armenia . . . . .
- [X] **AT** Austria . . . . .
- [X] **AU** Australia . . . . .
- [X] **AZ** Azerbaijan
- [X] **BA** Bosnia and Herzegovina . . . . .
- [X] **BB** Barbados
- [X] **BG** Bulgaria . . . . .
- [X] **BR** Brazil . . . . .
- [X] **BY** Belarus . . . . .
- [X] **CA** Canada
- [X] **CH and LI** Switzerland and Liechtenstein
- [X] **CN** China . . . . .
- [X] **CU** Cuba . . . . .
- [ ] **CZ** Czech Republic . . . . .
- [ ] **DE** Germany . . . . .
- [ ] **DK** Denmark . . . . .
- [ ] **EE** Estonia . . . . .
- [ ] **ES** Spain . . . . .
- [ ] **FI** Finland . . . . .
- [ ] **GB** United Kingdom
- [ ] **GD** Grenada
- [ ] **GE** Georgia . . . . .
- [ ] **GH** Ghana . . . . .
- [ ] **GM** Gambia
- [ ] **HR** Croatia . . . . .
- [ ] **HU** Hungary . . . . .
- [ ] **ID** Indonesia
- [ ] **IL** Israel . . . . .
- [ ] **IN** India . . . . .
- [ ] **IS** Iceland
- [ ] **JP** Japan . . . . .
- [ ] **KE** Kenya . . . . .
- [ ] **KG** Kyrgyzstan . . . . .
- [ ] **KP** Democratic People's Republic of Korea . . . . . .
- [ ] **KR** Republic of Korea . . . . .
- [ ] **KZ** Kazakhstan . . . . .
- [ ] **LC** Saint Lucia
- [ ] **LK** Sri Lanka
- [ ] **LR** Liberia
- [X] **LS** Lesotho . . . . .
- [X] **LT** Lithuania
- [X] **LU** Luxembourg
- [X] **LV** Latvia
- [X] **MD** Republic of Moldova . . . . .
- [X] **MG** Madagascar . . . . .
- [X] **MK** The former Yugoslav Republic of Macedonia . . . . . .
- [X] **MN** Mongolia
- [X] **MW** Malawi . . . . .
- [X] **MX** Mexico . . . . .
- [X] **NO** Norway
- [X] **NZ** New Zealand . . . . .
- [X] **PL** Poland . . . . .
- [ ] **PT** Portugal . . . . .
- [ ] **RO** Romania
- [ ] **RU** Russian Federation . . . . .
- [ ] **SD** Sudan
- [ ] **SE** Sweden
- [ ] **SG** Singapore
- [ ] **SI** Slovenia . . . . .
- [ ] **SK** Slovakia . . . . .
- [ ] **SL** Sierra Leone . . . . .
- [ ] **TJ** Tajikistan . . . . .
- [ ] **TM** Turkmenistan . . . . .
- [ ] **TR** Turkey . . . . .
- [ ] **TT** Trinidad and Tobago . . . . .
- [ ] **UA** Ukraine . . . . .
- [ ] **UG** Uganda . . . . .
- [ ] **US** United States of America . . . . . .
- [ ] **UZ** Uzbekistan . . . . .
- [ ] **VN** Viet Nam . . . . .
- [ ] **YU** Yugoslavia . . . . .
- [X] **ZA** South Africa . . . . .
- [ ] **ZW** Zimbabwe . . . . .

Check-boxes reserved for designating States which have become party to the PCT after issuance of this sheet:

- [ ] MA MORROCCO . . . . .
- [ ] TZ UNITED REPUBLIC OF TANZAWIA . . . . .

Precautionary Designation Statement: In addition to the designations made above, the applicant also makes under Rule 4.9(b) all other designations which would be permitted under the PCT except any designation(s) indicated in the Supplemental Box as being excluded from the scope of this statement. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn bythe applicant at the expiration of that time limit. *(Confirmation of a designation consists of the filing of a notice specifying that designation and the payment of the designation and confirmation fees. Confirmation must reach the receiving Office within the 15-month time limit.)*

Box No. VI PRIORITY CLAIM ☐ Further priority claims are indicated in the Supplemental Box.

Filing date of earlier application (day/month/year)	Number of earlier application	Where earlier application is: national application: country	regional application:* regional Office	international application: receiving Office
item (1) 10 December 1998	9827228.9	GB		
item (2)				
item (3)				

☐ The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) *(only if the earlier application was filed with the Office which for the purposes of the present international application is the receiving Office)* identified above as item(s): _____

* *Where the earlier application is an ARIPO application, it is mandatory to indicate in the Supplemental Box at least one country party to the Paris Convention for the Protection of Industrial Property for which that earlier application was filed (Rule 4.10(b)(ii)). See Supplemental Box.*

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) *(if two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used)*:

ISA / EP

Request to use results of earlier search; reference to that search *(if an earlier search has been carried out by or requested from the International Searching Authority)*:

Date *(day/month/year)* Number Country *(or regional Office)*

Box No. VIII CHECK LIST; LANGUAGE OF FILING

This international application contains the following **number of sheets**:

request	: 4
description (excluding sequence listing part)	: 20
claims	: 8
abstract	: 1
drawings	: 8
sequence listing part of description	:
Total number of sheets	: 41

This international application is **accompanied by** the item(s) marked below:

1. ☒ fee calculation sheet
2. ☐ separate signed power of attorney
3. ☐ copy of general power of attorney; reference number, if any:
4. ☐ statement explaining lack of signature
5. ☐ priority document(s) identified in Box No. VI as item(s):
6. ☐ translation of international application into (language):
7. ☐ separate indications concerning deposited microorganism or other biological material
8. ☐ nucleotide and/or amino acid sequence listing in computer readable form
9. ☐ other *(specify)*:

Figure of the drawings which should accompany the abstract:

Language of filing of the international application: ENGLISH

Box No. IX SIGNATURE OF APPLICANT OR AGENT

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

.....
BALDOCK: SHARON CLAIRE
AUTHORISED REPRESENTATIVE

For receiving Office use only

1. Date of actual receipt of the purported international application:
2. Drawings: ☐ received: ☐ not received:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:
4. Date of timely receipt of the required corrections under PCT Article 11(2):
5. International Searching Authority (if two or more are competent): ISA /
6. ☐ Transmittal of search copy delayed until search fee is paid.

For International Bureau use only

Date of receipt of the record copy by the International Bureau:

This sheet is not part of and does not count as a sheet of the international application.

PCT

FEE CALCULATION SHEET

Annex to the Request

For receiving Office use only

International application No.

Date stamp of the receiving Office

Applicant's or agent's file reference SCB/51598/001

Applicant
THE UNIVERSITY OF NOTTINGHAM

CALCULATION OF PRESCRIBED FEES

1. TRANSMITTAL FEE . . . . . 55 T

2. SEARCH FEE . . . . . 638 S

International search to be carried out by EP

(If two or more International Searching Authorities are competent in relation to the international application, indicate the name of the Authority which is chosen to carry out the international search.)

3. INTERNATIONAL FEE

Basic Fee

The international application contains 41 sheets.

first 30 sheets 285 b1

11 (remaining sheets) x 6 (additional amount) = 66 b2

Add amounts entered at b1 and b2 and enter total at B . . . . 351 B

Designation Fees

The international application contains ALL designations.

10 (number of designation fees payable *(maximum 10)*) x 65 (amount of designation fee) = 650 D

Add amounts entered at B and D and enter total at I . . . . . 1001 I

(Applicants from certain States are entitled to a reduction of 75% of the international fee. Where the applicant is (or all applicants are) so entitled, the total to be entered at I is 25% of the sum of the amounts entered at B and D.)

4. FEE FOR PRIORITY DOCUMENT *(if applicable)* . . . . . P

5. TOTAL FEES PAYABLE . . . . . 1694 TOTAL

Add amounts entered at T, S, I and P, and enter total in the TOTAL box

☐ The designation fees are not paid at this time.

MODE OF PAYMENT

☐ authorization to charge deposit account (see below)	☐ bank draft	☐ coupons
☒ cheque	☐ cash	☐ other *(specify)*:
☐ postal money order	☐ revenue stamps	

DEPOSIT ACCOUNT AUTHORIZATION *(this mode of payment may not be available at all receiving Offices)*

The RO/ _____

☐ is hereby authorized to charge the total fees indicated above to my deposit account.

☐ *(this check-box may be marked only if the conditions for deposit accounts of the receiving Office so permit)* is hereby authorized to charge any deficiency or credit any overpayment in the total fees indicated above to my deposit account.

☐ is hereby authorized to charge the fee for preparation and transmittal of the priority document to the International Bureau of WIPO to my deposit account.

Deposit Account No. | Date *(day/month/year)* | Signature

See Notes to the fee calculation sheet

WIPO International Bureau
34 Chemin des Colombettes
CH-1211 Geneva 20
SWITZERLAND

30 May 2001

BY FAX TO: 00 41 22 733 5428
FROM: 020 7430 7600 - 1 PAGE(S)
CONFIRMATION BY POST

Dear Sirs,

International Patent Application No. PCT/GB99/04182
THE UNIVERSITY OF NOTTINGHAM et al
Our Ref: SCB/NLW/51598/001

On behalf of the applicants, we hereby request a change in the names of the applicants on the request in accordance with PCT Rule 92 bis.1(a).

We advise that the applicant/inventor Price; Michael Rawling is deceased. We therefore request recordal of the change in the person of the applicant as follows:

Price; Frances, Legal representative of Price; Michael Rawling (deceased)
351 Derby Road
Nottingham
United Kingdom

State of Nationality: United Kingdom
State of Residence: United Kingdom

We look forward to receiving notification that this change has been recorded. If any further information is required in order to record the change we request that you contact the named representative.

Yours faithfully,

Baldock; Sharon Claire
BOULT WADE TENNANT
: 297609: NLW: KMB: LONDOCS

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : G01N 33/574, C07K 14/47, G01N 33/564, C12N 5/06 — A1

(11) International Publication Number: **WO 00/34787**

(43) International Publication Date: 15 June 2000 (15.06.00)

(21) International Application Number: PCT/GB99/04182

(22) International Filing Date: 10 December 1999 (10.12.99)

(30) Priority Data:
9827228.9 10 December 1998 (10.12.98) GB

(71) Applicant *(for all designated States except US)*: THE UNIVERSITY OF NOTTINGHAM [GB/GB]; University Park, Nottingham NG7 2RD (GB).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: ROBERTSON, John, Russell [GB/GB]; 16 Barratt Lane, Attenborough, Nottingham NG9 6AF (GB). GRAVES, Catherine, Rosamund, Louise [GB/GB]; 6 Lindy Close, Kinoulton, Nottingham NG7 3RD (GB). PRICE, Michael, Rawling [GB/GB]; 351 Derby Road, Nottingham (GB).

(74) Agent: BOULT WADE TENNANT; Verulam Gardens, 70 Gray's Inn Road, London WC1X 8BT (GB).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: CANCER DETECTION METHOD AND REAGENTS

(57) Abstract

Sensitive and specific methods are provided for use in detecting the presence of cancer marker proteins in the body fluids of a mammal. Also provided are autoantibodies for use in these methods, and immortalized cells which are a source of the autoantibodies.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
AM	Armenia	**FI**	Finland	**LT**	Lithuania	**SK**	Slovakia
AT	Austria	**FR**	France	**LU**	Luxembourg	**SN**	Senegal
AU	Australia	**GA**	Gabon	**LV**	Latvia	**SZ**	Swaziland
AZ	Azerbaijan	**GB**	United Kingdom	**MC**	Monaco	**TD**	Chad
BA	Bosnia and Herzegovina	**GE**	Georgia	**MD**	Republic of Moldova	**TG**	Togo
BB	Barbados	**GH**	Ghana	**MG**	Madagascar	**TJ**	Tajikistan
BE	Belgium	**GN**	Guinea	**MK**	The former Yugoslav Republic of Macedonia	**TM**	Turkmenistan
BF	Burkina Faso	**GR**	Greece			**TR**	Turkey
BG	Bulgaria	**HU**	Hungary	**ML**	Mali	**TT**	Trinidad and Tobago
BJ	Benin	**IE**	Ireland	**MN**	Mongolia	**UA**	Ukraine
BR	Brazil	**IL**	Israel	**MR**	Mauritania	**UG**	Uganda
BY	Belarus	**IS**	Iceland	**MW**	Malawi	**US**	United States of America
CA	Canada	**IT**	Italy	**MX**	Mexico	**UZ**	Uzbekistan
CF	Central African Republic	**JP**	Japan	**NE**	Niger	**VN**	Viet Nam
CG	Congo	**KE**	Kenya	**NL**	Netherlands	**YU**	Yugoslavia
CH	Switzerland	**KG**	Kyrgyzstan	**NO**	Norway	**ZW**	Zimbabwe
CI	Côte d'Ivoire	**KP**	Democratic People's Republic of Korea	**NZ**	New Zealand		
CM	Cameroon			**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
CU	Cuba	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

CANCER DETECTION METHOD AND REAGENTS

The present invention relates to highly sensitive and specific methods for detecting the presence of cancer marker proteins in the bodily fluids of a mammal, to autoantibodies for use in these methods, to immortalised cells for obtaining these autoantibodies and to kits for performing the methods. These methods are useful in the early detection of carcinogenic or pre-neoplastic modifications in asymptomatic patients, in monitoring the progress of cancer, in screening for recurrence of the disease in patients who have previously undergone anti-cancer treatment, in monitoring the efficacy of a systematic treatment in a patient and in determining the most appropriate treatment for a particular patient.

Cancer and pre-neoplastic cells are characterised by the production of cancer-associated marker proteins. These often consist of aberrant forms of wild-type proteins, which are produced by cancer cells as a result of genetic mutations or altered post-translational processing. Alternatively, cancer markers can also be proteins that become over-expressed in tumour cells, usually as a result of gene amplification or abnormal transcriptional regulation. In some cases, these two phenomena may occur at the same time leading to an accumulation of modified proteins throughout the development of the disease. For example, modified forms of Ras, p53, c-myc, MUC-1, c-erbβ2 have been found to be associated with a wide variety of cancers.

Cancer associated proteins are found both in the tissues and in the bodily fluids of an individual who carries pre-neoplastic or cancer cells. Their levels are very low at the early stages of the carcinogenic

process and increases during progression of the disease. The detection of these proteins has advantageously been used in routine tests for the diagnosis of cancer but, unfortunately, these assays have many limitations. In particular, commercial antibodies available for use in standard tests are usually not sensitive enough to detect the low levels of cancer-associated proteins that are found at the very early stages of the disease, for example in asymptomatic patients, when a treatment would be the most effective. In addition, most commercial antibodies are not specific for modified forms of cancer-associated markers and cross-react with wild-type forms of these proteins. As a consequence, they are only useful for detecting substantial increases in serum levels of cancer marker proteins, which usually occur at advanced stages of cancer.

For example, the commercial assay CA15-3, which detects both unmodified and modified forms of MUC1, is useful in the diagnosis of metastatic breast cancers, which are characterised by elevated serum levels of MUC1. However, this assay cannot be used in screening for neoplasia or primary breast cancer because the serum levels of MUC1 at these stages do not differ significantly from those in normal individuals (Robertson *et al.* (1990), Eur. J. Cancer **26**: 1127-1132). Other marker proteins such as, for example, carcinoembryonic antigen (CEA) and the marker CA19.9 have been reported to be elevated in the serum of patients with metastatic breast and colorectal cancer but not that of patients with primary cancers (Robertson *et al.* (1991), Cancer Immunol. Immunother. **133**: 403-410; Thomas *et al.* (1991) Br. J. Cancer **63**: 975-976). Also in the case of these cancer markers, available commercial assays are not able to discriminate between modified and wild-type forms of

the proteins and are therefore of limited use . Furthermore, commercially available antibodies, by cross-reacting with normal forms of cancer-associated proteins, may also lead to false positive results. Thus, there is a need in the art for more sensitive and specific antibodies to use in these assays in order to detect pre-neoplastic and early carcinogenic modifications.

As used herein the terms "cancer-associated marker protein", "cancer-associated protein", "marker protein" or "cancer marker" all refer to cancer-associated modified forms of wild-type proteins.

Cancer markers often differ from the corresponding wild-type proteins in such a way that they are recognised as foreign molecules by the immune system of an individual, triggering an autoimmune-response. The immune-response may be humoral, leading to the production of autoantibodies against the cancer marker protein. Autoantibodies are naturally occurring antibodies directed to an antigen that an individual's immune system recognises as foreign even though that antigen actually originated in that individual. For example, modified forms of p53, MUC-1, c-myc, c-erb3 and Ras proteins may elicit production of autoantibodies. As used herein the term "autoantibody" refers to an antibody directed against a self-originating antigen, which antibody is naturally occurring in the circulation of an individual or to an antibody which exhibits the characteristics of the naturally occurring antibody in that it recognises the said self-originated antigen but which is produced outside the body, for example, by an immortalised cell.

As will be described in the Examples below, the present inventors have surprisingly found that autoantibodies produced by patients suffering from

cancer specifically recognise cancer-associated marker proteins from the same patients or from other patients with cancer and show very low cross-reactivity with wild-type forms of these proteins. Furthermore, the present inventors have found that the above autoantibodies have a much higher sensitivity than the antibodies currently used in routine tests and are therefore unable to detect smaller quantities of cancer-associated marker proteins. Autoantibodies produced by patients with cancer may therefore be used to design alternative, more reliable and sensitive tests to detect pre-neoplastic or carcinogenic modifications in an individual from the very beginning of their occurrence. These assays may also be employed to detect cancer or pre-neoplasia in any other mammal, by utilising autoantibodies produced by a mammal from the same species as the one to be tested or autoantibodies having the same characteristics as such.

The present invention provides a more sensitive and specific assay system for the detection of pre-neoplasia or cancer in a mammal, which allows the detection of cancer-associated marker proteins from the early stages of the disease.

Accordingly, in a first aspect the invention provides an *in vitro* method for detecting a cancer-associated marker protein present in a bodily fluid of a mammal which method comprises the steps of:

(a) contacting a sample of bodily fluid from said mammal with antibodies directed against at least one epitope of said marker protein; and

(b) detecting the presence of any complexes formed between said antibodies and any marker protein present in said sample;

wherein said antibodies are mammalian autoantibodies to said cancer-associated marker protein which are derived from the same species as the mammal from which said sample has been obtained.

The presence of said complexes is indicative of the presence of cancer associated marker proteins in said mammal.

As used herein "derived" means an autoantibody or autoantibodies isolated from the said species or an autoantibody or autoantibodies having the characteristics of an autoantibody or autoantibodies isolated from said species.

The method of the invention may employ a single autoantibody directed against a particular cancer marker protein. Alternatively, a panel of autoantibodies recognising a number of cancer-associated proteins may be utilised in order to obtain a profile of cancer markers present in a particular individual. This leads to a more reliable diagnosis and provides information useful in the choice of the most appropriate treatment for an individual.

The assay method of the invention is performed on a sample of a biological fluid from the patient such as, for example, plasma, serum, whole blood, urine, lymph, faeces, cerebrospinal fluid or nipple aspirate, depending of the nature of the cancer to be detected. Since it is non-invasive the assay can be repeated as often as it is necessary to screen for early neoplastic or carcinogenic modifications, to follow the development of the disease, to test for recurrence of the disease, to verify the efficacy of a treatment or to select the most appropriate treatment for a particular patient.

The method of the invention can be performed using any immunological technique known to those skilled in the art of immunochemistry. As examples, ELISA, radio immunoassays or similar techniques may be utilised. In general, an appropriate autoantibody is immobilised on a solid surface and the sample to be tested is brought into contact with the autoantibody. If the cancer marker protein recognised by the autoantibody is present in the sample, a complex autoantibody-marker is formed. The complex can then be directed or quantitatively measured using, for example, a labelled secondary antibody which specifically recognises an epitope of the marker protein. The secondary antibody may be labelled with biochemical markers such as, for example, horseradish peroxidase (HRP) or alkaline phosphatase (AP), and detection of the complex can be achieved by the addition of a substrate for the enzyme which generates a colorimetric, chemiluminescent or fluorescent product. Alternatively, the presence of the complex may be determined by addition of a marker protein labelled with a detectable label, for example an appropriate enzyme. In this case, the amount of enzymatic activity measured is inversely proportional to the quantity of complex formed and a negative control is needed as a reference to determine the presence of antigen in the sample. Another method for detecting the complex may utilise antibodies or antigens that have been labelled with radioisotopes followed by measure of radioactivity.

The method of the invention can be performed in a qualitative format, which determines the presence or absence of a cancer marker protein in the sample or in a quantitative format, which, in addition, provides a measurement of the quantity of cancer marker protein present in the sample. The quantity of marker protein present in a sample may be calculated utilising any of

the above described techniques. In this case, prior to performing the assay, it is necessary to draw a standard curve by measuring the signal obtained, using the same detection reaction that will be used for the assay, from a series of standard samples containing known concentrations of the cancer marker protein. The quantity of cancer marker present in a sample to be screened is then interpolated from the standard curve.

If it is necessary to verify the presence of a number of cancer marker proteins in a sample, the assay of invention may be performed in a multi-well assay plate where each of the different autoantibodies utilised is placed in a different well.

The method of the invention can be employed in a variety of clinical situations such as, for example, in the assessment of the predisposition of an individual towards the development of a cancer, in the detection of pre-neoplastic or carcinogenic modifications in asymptomatic patients, in the diagnosis of primary or secondary cancer, in monitoring the progression of the disease in a patient, in screening for recurrence of carcinogenic modifications in a patient who has previously been diagnosed as carrying cancer cells and has undergone a therapy to reduce the number of these cells or in the choice of the more appropriate anti-cancer treatment for a patient suffering from cancer. The method of the invention is also suitable for veterinary use in the same clinical situations as the ones described above.

The assay method of the invention may be employed to detect cancer marker proteins that are associated with a variety of cancers such as, for example, lymphomas, leukaemia, breast cancers, colorectal cancers, lung cancers, pancreatic cancers, prostate cancers, cervical cancers, ovarian cancers, endometrial cancers and cancers of the skin. The method of the invention is particularly suitable to

detect and monitor primary breast cancer (PBC) and advanced breast cancer (ABC).

In a second aspect the invention provides autoantibodies and reagents comprising said autoantibodies for use in the assay, which specifically recognise at least one epitope of a mammalian cancer-associated marker protein. Such autoantibodies may be isolated from the blood or peripheral blood monocytes of such a mammal, preferably a human. Alternatively, the autoantibodies can be produced by immortalised B lymphocytes and directed to an antigen originated in the mammal itself. The reagents comprising autoantibodies according to this aspect of the invention are particularly suitable for use in the detection of mammalian cancer-associated marker proteins in body fluids. Preferred autoantibodies to use in the assay include those against cancer-associated forms of the glycoprotein MUC1 (Batra, SK. *et al.* (1992) Int J. Pancreatology **12**: 271-283), the signal transduction/ cell cycle regulatory protein c-myc (Blackwood, E. M. *et al* . (1994) Molecular Biology of the Cell **5**: 597-609), p53 (Matlashewski, G. *et al.* (1984) EMBO J. **3**: 3257-3262), c-erbβ2 (Dsouza, B. *et al.* (1993) Oncogene **8**: 1797-1806) and Ras (Gnudi, L. *et al.* (1997) Mol. Endocrinol. **11**: 67-76). However, autoantibodies against any other cancer-associated marker protein may be employed in the assay. Particularly suitable for the detection of breast cancers are autoantibodies against a modified MUC1, BRCA1, BRCA2, p53, c-myc,c-erbβ2 or Ras protein associated with primary breast cancer and autoantibodies against a modified MUC1, BRCA1, BRCA2 p53, c-myc,cerbβ2 or Ras protein associated with advanced breast cancer. These autoantibodies are

preferably derived from patients diagnosed with the same type of cancer as the one to which these cancer marker protein are associated.

The invention also provides immortalised cell populations capable of producing the above autoantibodies.

The cell populations of the invention may be produced by any method known in the art. As will be described in detail in Example 1 below, B cells from patients diagnosed with cancer may be, for example, immortalised with Epstein Barr Virus. ELISA or any similar techniques may be performed to screen for the production of autoantibodies, utilising marker proteins obtained from a patient affected from cancer which have been immobilised on a solid support.

The invention further provides kits for detecting one or more cancer-associated marker proteins in the biological fluids of a mammal. Such kits include at least mammalian autoantibodies directed against one or more epitopes of a cancer-associated marker protein and means for detecting the formation of complexes between the autoantibodies and the cancer-associated marker protein. Preferably, the autoantibodies are immobilised on a solid surface.

The present invention will be further understood with reference to the following Examples and to the accompanying Figures in which:

Figure 1 shows the results of an ELISA assay to examine the reactivity of autoantibodies produced by B cells derived from six patients diagnosed with breast cancer (1 to 4, with primary breast cancer, 7 and 11 with advanced breast cancer). For each group of autoantibodies, MUC1 protein purified from the

same patient from which the B cells were taken, from other patients or from normal subjects was used as immobilised antigens. The reactivity of mouse monoclonal B55 anti-MUC1 antibody in a parallel assay is included as a comparative control. PBS or antibodies produced by B lymphocytes derived from four healthy subject (N10, N12, N13 and N14) are used as negative controls. MUC1 was eluted from immunoaffinity columns using 0.25 M glycine pH 2.5.

Figure 2 shows the results of an ELISA assay to assess the reactivity of autoantibodies obtained from B cells derived from patients diagnosed with primary breast cancer with MUC1 protein from different sources. The reactivity of B55 is included as a comparative control. PBS is used as a negative control.

Figure 3 shows the results of a surface plasmon resonance experiment to measure the binding of autoantibodies produced by B cells derived from patients diagnosed with primary breast cancer to MUC1 protein isolated (a) from the serum of patients with advanced breast cancer or (b) from the urine of normal individuals.

Figure 4 shows the sequence of the peptide that was used to immunoaffinity-purify MUC1 antibodies from the sera of patients with advanced breast cancer.

Figure 5 shows the results of an ELISA assay employing immobilised autoantibodies from a patient with (2) primary breast cancer or (3) advanced breast cancer to detect MUC1 protein purified from the serum of a patient diagnosed with advanced breast cancer or from the urine of a healthy individual. The result of a parallel utilising the anti-MUC1 C595 antibody

(1) is included as a comparative example.

Figure 6 shows the results of an ELISA assay utilising immobilised autoantibodies from the B cells of patients with primary breast cancer to detect MUC1 protein in serum samples from healthy individuals or from patients diagnosed with primary or advanced breast cancer. The results obtained with the C595 antibody in a parallel assay are included as comparative examples.

Figure 7 shows the results of an ELISA assay using immobilised autoantibodies from the B cells of patients with primary breast cancer to detect MUC1 protein in sequential serum samples from a patient with advanced breast cancer throughout the progression of the disease. The results obtained with the monoclonal C595 antibody in a parallel assay or with the commercial CA15-3 assay are included as comparative examples.

Figure 8 shows the results of a number of determinations of the reactivity of sera from breast cancer patients with ABC MUC1 and urinary MUC1.

Example 1: Immortalisation of mononucleocytes.

Peripheral blood mononucleocytes were purified from a 4ml sample of heparinised blood from patients or normal individuals using lymphocyte separation medium (ICN flow), as described in detail in the manufacturers instructions. Isolated mononucleocytes were washed in PBS and resuspended in 1 ml of a semipurified preparation of Epstein Barr Virus (EBV) from the B95-8 marmoset transformed leukocyte EBV-producing cell line. The cells were then incubated for 1 hour at 37°C in 5% CO_2 and centrifuged at 17000 rpm. The EBV supernatant was removed and the

mononucleocytes were washed three times with RPMI medium, resuspended in RPMI medium supplemented with 10% fetal bovine serum and 5μg/ml phytoheamatagglutinin (PHA-P) and seeded in multi-wells tissue culture plates. The medium was changed every 3 days and used as a source of autoantibodies.

Example 2: Assessment of the reactivity of autoantibodies with MUC1 antigen from different sources

Methods:

1) Immunoaffinity purification of MUC1 antigen

MUC1 was purified from the serum of patients diagnosed with primary breast cancer or advanced breast cancer or from the urine of healthy subjects according to the following protocol.

The mouse monoclonal B55 antibody (also known as NCRC 11 as described by Ellis et al. (1984) Histopathology 8: 501-516 and in International Patent Application No. WO 89/01153) was conjugated to CNBr sepharose beads. Serum or urine samples were diluted 1/10 in PBS and incubated with the antibody conjugated sepharose beads overnight at 4°C with rolling. The beads were centrifuged and the supernatant removed. In order to remove any molecule non-specifically bound to the beads, these were washed in PBS for 5 times or until the washing buffer showed no absorbance at 280nm. Each wash was performed by resuspending the beads in PBS, rolling for 10 minutes, centrifuging and removing the supernatant. The washed beads were resuspended in 0.25 M glycine pH 2.5, rolled at room temperature for 10 minutes and centrifuged. The supernatant was removed, adjusted to pH 7 by addition of TRIS and stored at 4°C labelled "glycine fraction". The beads were then resuspended

in 25mM diethylamine (DEA) pH 11, rolled at room temperature for 10 minutes and centrifuged. The supernatant was again removed, adjusted to pH 7 by addition of TRIS and stored at 4°C labelled "25 DEA fraction". The beads were finally resuspended in 100mM DEA pH 11, rolled at room temperature for 10 minutes and centrifuged. The supernatant was removed, adjusted to pH 7 by addition of TRIS and stored at 4°C labelled "100 DEA fraction". The presence of MUC1 in the three fractions were confirmed by ELISA using the monoclonal antibody B55 or C595 (also known as NCRC, available from the Cancer Research Campaign). In order to remove contaminating immunoglobulins, fractions were incubated with DTT (to 50mM) for 30 minutes, then iodacetamide (to 75mM) before being subjected to gel filtration on a S300 column. Fractions were assayed for MUC1 content by ELISA. MUC1 containing fractions are titrated so as to give equivalent absorbances to previous batches.

2) ELISA assay

Different MUC1 preparations, obtained as described above, were appropriately diluted with PBS and plated out at 50μl per well in a 96 well microtitre assay plate and left to dry overnight. The plate was then washed once with PBS/Tween to remove residual salt crystals, blocked for 60 minutes with a fresh solution of 2% (w/v)polyvinylpyrrolidone (PVP) in PBS and washed three times with PBS/Tween. Culture supernatant of immortalised lymphocytes derived from patients diagnosed with primary or secondary breast cancer were plated out in triplicate, at 50μl per well. As a comparative control the mouse monoclonal anti-MUC1 antibody B55 was also plated in triplicate. The plate was incubated for 60 minutes at room temperature with shaking and washed four times with PNS/Tween. 50μl of HRP conjugated anti-human or anti-

mouse secondary antibody (obtained from Dako) were added to each well at the dilution recommended by the manufacturer, and incubated for 60 minutes at room temperature with shaking. The plate was then washed again four times with PBS/Tween. 50μl of TetraMethylBenzidine (TMB) were added to each well and optical density (OD) at 650nm for each well of the assay plate was read kinetically over a period of 10 minutes.

Results:

Figure 1 shows the result of an ELISA assay to assess the reactivity of autoantibodies produced by lymphocytes derived from six patients diagnosed with breast cancer (1 to 4, with primary breast cancer, 7 and 11 with advanced breast cancer) with MUC1 protein purified from the same patient from which the antibody was taken, from other patients or from healthy subjects. The healthy subjects used in this study were women who had no clinical and/or mammographical evidence of breast cancer. The reactivity of the monoclonal anti-MUC1 B55 antibody was measured as a comparative control. Antibodies produced by lymphocytes from four healthy subjects (N10 to N14) were used as a negative control.

The results presented demonstrate that B lymphocytes derived from patients with breast cancer produce autoantibodies that are able to recognise MUC1 protein isolated both from the same and from different patients. In addition, these autoantibodies bind with high specificity to MUC1 present in patients with cancer, showing almost no reactivity with MUC1 isolated from healthy individuals. These results are highly reproducible, since different autoantibodies show a very similar reactivity profile with MUC1 protein purified from different sources. Furthermore, the results obtained also indicate that

the sensitivity of the autoantibodies for cancer-associated MUC1 is much greater than that observed for the monoclonal B55 antibody. Furthermore, antibodies produced by lymphocytes from normal patients did not show this profile.

Figure 2 shows the reactivity of autoantibodies secreted by immortalised B lymphocytes derived from patients with primary breast cancer with MUC1 protein from different sources, compared with that of B55. The profile of reactivity of the different autoantibodies is again very reproducible. The autoantibodies show high specificity for MUC1 present in the serum of patients with cancer and have almost no affinity for MUC1 isolated from healthy individuals or from the breast cancer cell line ZR75-1. Furthermore, the affinity of the autoantibodies for MUC1 protein associated with either primary breast cancer or advanced breast cancer is much higher that measured for B55.

Example 3: Measure of the affinity of autoantibodies with Surface Plasmon Resonance.

Methods

Surface Plasmon Resonance was performed on Iasys Biosensor Plus (from Affinity Sensor). MUC1 protein from patients with advanced breast cancer and from normal individuals were adhered to amino silane coated cells following the manufacturers instructions and the cells were blocked with 1% (w/v)poly-vinylpyrrolidone (PVP). Control cells coated only with 1% PVP were also produced. The binding of different dilutions of culture supernatant derived from B cells from patients with primary breast cancer was measured using the following experimental conditions:

Sampling interval:	0.3 msecs
Stirrer speed:	70rpm
Temperature:	24°C
Binding Time:	3 min
Dissociation with PBS:	2 minutes
Regeneration with 20mM Hcl:	3 minutes
Re-equilibration with PBS:	5 minutes

Results

Figure 3 shows that the autoantibodies produced by B lymphocytes derived from a patient with primary breast cancer bind with a much higher affinity to MUC1 isolated from another patient with breast cancer than MUC1 isolated from a healthy individual.

Example 4: Detection of MUC1 antigen in ELISA assays utilising autoantibodies.

Method:

1) Purification of anti-MUC1 autoantibodies from sera

The MUC1 peptide TAP2, with the sequence shown in Figure 4, was conjugated to CNBr-sepharose beads. Pooled sera from patients diagnosed with advanced breast cancer were diluted 1/10 in PBS and were incubated with the conjugated sepharose beads overnight at 4°C with rolling (in the ratio of 25ml of serum to 1ml of beads). After centrifugation the supernatant was removed and the beads were washed 5 times with PBS or until absorbance at 280nm was zero. Each wash was performed by resuspending the beads in PBS, rolling for 10 minutes, centrifuging and removing the supernatant. The beads were resuspended in 1ml of 3M sodium thiocyanate in PBS, rolled at room temperature for 10 minutes and centrifuged. The supernatant was removed and dialysed against PBS at 4°C. The anti-MUC1 content was then confirmed by ELISA using as immobilised antigen both MCU1 isolated from

patients with advanced breast cancer and a MUC1 peptide, with sequence APDTRTPAPG and conjugated to BSA.

2) Biotinylation of anti-MUC1 autoantibodies

The autoantibodies obtained as described above were concentrated to a volume of 100μl by using centrifugal filters and then diluted to a volume of 1ml with 0.1 sodium tetraborate buffer pH 8.8. 20μg of N-hydroxysuccinimide biotin were added and the autoantibodies/biotin solution was incubated for 4 hours at room temperature with rolling. The reaction was stopped by addition of 10μl of 1M NH_4Cl and incubation for ten minutes. The autoantibodies were then dialysed against PBS for thirty-six hours at 4°C to remove unbound biotin. Aliquots of the autoantibodies solution were frozen and stored at -20°C in the dark until use.

3) ELISA ASSAY

Culture supernatant of lymphocytes derived from patients with primary breast cancer or advanced breast cancer or the monoclonal anti-MUC1 C595 antibody were plated out at 50μl per well in a 96 well microtitre assay plate and incubated overnight at 4°C. The plate was then washed 4 times with PBS/Tween, blocked for 60 minutes with a fresh solution of 2% (w/v)polyvinylpyrrolidone (PVP)in PBS and washed twice with PBS/Tween. 50μl per well of MUC1 from different sources were added. After incubation at room temperature for sixty minutes, the plate was washed again four times with PBS/Tween. 50μl of the appropriate biotinylated secondary antibody, either C595 or autoantibody purified from a pool of sera from a patient with advanced breast cancer, prepared as described above, were added to each well and incubated for 60 minutes at room

temperature. After 4 washes with PBS/Tween, 50μl of streptavidin-HRP were added to each well and incubated at room temperature for 60 minutes. The plate was again washed four times, 50μl of TMB were added to each well and optical density (OD) at 650nm for each well of the assay plate was read kinetically over a period of 10 minutes.

Results:

Figure 5 shows the results of an ELISA assay utilising as immobilised antibodies autoantibodies produced by B lymphocytes derived from patients with primary or advanced breast cancer, compared with those obtained in a parallel assay with the monoclonal anti-MUC1 C595 antibody. The data indicate that autoantibodies from patients with breast cancer can be used in ELISA assays to specifically detect modified forms of MUC1 protein associated with cancer. These assays are more sensitive and show higher specificity than those utilising the monoclonal antibody C595.

Example 5: Use of the assay to detect MUC1 proteins in serum samples of patients.

An ELISA assay was performed, as described in Example 4, on serum samples from healthy individuals or patients with primary or advanced breast cancer utilising as immobilised antibodies the autoantibodies produced by B lymphocytes derived from patients with primary breast cancer. A parallel assay utilising the monoclonal anti-MUC1 antibody C595 was performed on the same samples. The results, shown in Figure 6, indicate that the assay employing autoantibodies is able to detect with high sensitivity MUC1 circulating in the blood of patients with breast cancer. In addition, contrary to utilising the monoclonal antibody C595, this assay

has a very high specificity for cancer-associated forms of MUC1.

Example 6: Use of the assay to monitor the progression of the disease.

An ELISA assay was performed, as described in Example 4, on sequential serum samples from a patient diagnosed with metastatic cancer throughout the progression of the disease, using as immobilised antibodies the autoantibodies produced by B lymphocytes derived from patients with primary breast cancer or the monoclonal anti-MUC1 C595 antibody. The commercial assay CA15-3 was also performed on the same samples. Figure 7 shows that the assay employing autoantibodies can be used to follow the progression of cancer in a patient, wherein increasing levels of MUC1 detected in the assay indicate exacerbation of the disease. The data also demonstrate that the use of autoantibodies leads to results that better represent the development of the disease than those obtained with either the C959 antibody or the CA15-3 assay.

Example 7: Comparison of the specificity of anti-MUC1 autoantibodies to urinary or ABC MUC1

Method:

Preparations of ABC MUC1 (MUC1 isolated from the serum of patients diagnosed with advanced breast cancer) and urinary MUC1 were prepared as described in Example 2.

Aliquots of the ABC and urinary MUC1 preparations were dried onto the wells microtitre plates separately at concentrations giving equivalent NCRC-11 binding. After blocking with 2% PVP, serum samples taken from patients with breast cancer, diluted 1/100 with PBS, were added to the wells and

any anti-MUC1 antibodies in the sera allowed to bind. After washing, the bound antibodies were probed with anti-human IgM-HRP and anti-human IgG-HRP conjugates.

Results

Figure 8 shows the results of a number of determinations of reactivity of sera from breast cancer patients with ABC and urinary MUC1. Sera from the majority of patients clearly exhibit greater specificity for the ABC MUC1 as compared to urinary MUC1.

CLAIMS:

1. An *in vitro* method for detecting a cancer-associated marker protein present in a bodily fluid of a mammal which method comprises the steps of:

(a) contacting a sample of bodily fluid from said mammal with antibodies directed against at least one epitope of said marker protein; and

(b) detecting the presence of any complexes formed between said antibodies and any marker protein present in said sample;

wherein said antibodies are mammalian autoantibodies to said cancer-associated marker protein which are derived from the same species as the mammal from which said sample has been obtained.

2. A method as claimed in claim 1 wherein said sample is from a mammal substantially asymptomatic for pre-neoplasia or cancer.

3. A method as claimed in claim 1 wherein said sample is from a mammal symptomatic for cancer.

4. A method as claimed in claim 1 wherein said sample is from a mammal which has received therapy for cancer.

5. A method as claimed in any preceding claim wherein the mammal is a human and the autoantibodies are human autoantibodies.

6. A method as claimed in any preceding claim

wherein said bodily fluid is plasma, serum, whole blood, urine, faeces, lymph, cerebrospinal fluid or nipple aspirate.

7. A method as claimed in any preceding claim wherein said cancer-associated marker protein is associated with lymphomas, leukaemias, breast cancers, colorectal cancers, lung cancers, pancreatic cancers, prostate cancers, cervical cancers, ovarian cancers, endometrial cancers or cancers of the skin.

8. A method as claimed in claim 7 wherein said cancer-associated marker protein is a breast cancer-associated marker protein.

9. A method as claimed in any preceding claim wherein said cancer-associated marker protein is a modified MUC1, BRCA1, p53, c-myc c-erbβ2 or Ras protein.

10. A method as claimed in claim 8 wherein said cancer-associated marker protein is a modified MUC1, BRCA1, BRCA2, p53, c-myc, c-erbβ2 or Ras protein associated with primary breast cancer.

11. A method as claimed in claim 8 wherein said cancer-associated marker protein is a modified MUC1, BRCA1, BRCA2, p53, c-myc, c-erbβ2 or Ras protein associated with advanced breast cancer.

12. A method as claimed in claim 10 wherein said autoantibodies are obtainable from monocytes isolated from a patient with primary breast cancer.

13. A method as claimed in claim 11 wherein said autoantibodies are obtainable from monocytes isolated from a patient with advanced breast cancer.

14. A method as claimed in any preceding claim wherein said autoantibodies are produced by an immortalized cell or cell population.

15. A method as claimed in any one of claims 1 to 14 wherein said autoantibodies are polyclonal antibodies.

16. A method as claimed in any preceding claim wherein said autoantibodies are immobilized on a solid surface.

17. A method as claimed in claim 16 wherein any complexes formed between said autoantibodies and any cancer-associated marker protein present in said sample are detected using secondary antibodies or autoantibodies specific for at least one epitope of said marker protein, said secondary autoantibodies carrying a detectable label.

18. A method as claimed in claim 16 wherein in addition to said sample a labelled cancer-associated marker protein is added carrying at least one epitope recognised by said autoantibodies.

19. Use of a method as claimed in any one of claims 1 to 18 to screen for recurrence of cancer after a treatment, to monitor systemic therapies or to select therapies.

20. A diagnostic reagent which comprises mammalian autoantibodies with a specificity for at least one epitope of a mammalian cancer-associated marker protein.

21. A diagnostic reagent as claimed in claim 20 for use in detecting the presence of a mammalian

cancer-associated marker protein in a sample of body fluid.

22. A reagent as claimed in claim 20 or claim 21 wherein said autoantibodies are human autoantibodies and said marker protein is a human cancer-associated marker protein.

23. A reagent as claimed in any one of claims 21 or 22 wherein said autoantibodies have specificity for at least one epitope of a cancer-associated marker protein associated with lymphomas, leukaemias, breast cancers, colorectal cancers, lung cancers, pancreatic cancers, prostate cancers, cervical cancers, ovarian cancers, endometrial cancers or cancers of the skin.

24. A reagent as claimed in claim 23 wherein said autoantibodies have specificity for at least one epitope of a breast cancer-associated marker protein.

25. A reagent as claimed in any one of claims 20 to 24 wherein said marker protein is a modified MUC1, BRCA1, BRCA2, p53, c-myc, c-erbβ2 or Ras protein.

26. A reagent as claimed in claim 24 wherein said marker protein is a modified MUC1, BRCA1, BRCA2, p53, c-myc, c-erbβ2 or Ras protein associated with primary breast cancer.

27. A reagent as claimed in claim 24 wherein said marker protein is a modified MUC1, BRCA1, BRCA2, p53, c-myc, c-erbβ2 or Ras protein associated with advanced breast cancer.

28. A reagent as claimed in claim 26 wherein

said autoantibodies are obtainable from monocytes isolated from a patient with primary breast cancer.

29. A reagent as claimed in claim 27 wherein said autoantibodies are obtainable from monocytes isolated from a patient with advanced breast cancer.

30. An immortalized cell population capable of producing autoantibodies directed against at least one epitope of a mammalian cancer-associated marker protein.

31. An immortalized cell population as claimed in claim 30 which is capable of producing autoantibodies directed against at least one epitope of a human cancer-associated marker protein.

32. An immortalized cell population as claimed in claim 31 or claim 32 wherein said autoantibodies are directed against at least one epitope of a cancer-associated marker protein associated with lymphomas, leukaemias, breast cancers, colorectal cancers, lung cancer, pancreatic cancers, prostate cancers, cervical cancers, ovarian cancers, endometrial cancers or cancers of the skin.

33. An immortalised cell population as claimed in claim 32 wherein said autoantibodies are directed against an epitope of a breast cancer-associated marker protein.

34. An immortalized cell population as claimed in any one of claims 31 to 33 wherein said autoantibodies are directed against a modified MUC1, BRCA1, BRCA2, p53, c-myc, c-erbβ2 or Ras protein.

35. An immortalized cell population as claimed

in claim 33 wherein said autoantibodies are autoantibodies to a modified MUC1, BRCA1, BRCA2, c-myc, p53, c-erbβ2 or Ras protein associated with primary breast cancer.

36. An immortalized cell population as claimed in claim 33 wherein said autoantibodies are autoantibodies to a modified MUC1, BRCA1, BRCA2, c-myc, c-erbβ2 or Ras protein associated with advanced breast cancer.

37. An immortalized cell population as claimed in anyone of claims 30 to 36 which is derived from monocytes isolated from a patient or a group of patients having cancer or other neoplasia.

38. An immortalised cell population as claimed in claim 35 wherein said cell population is derived from monocytes of a patient or group of patients having primary breast cancer.

39. An immortalised cell population as claimed in claim 36 wherein said cell population is derived from monocytes of a patient or group of patients with advanced breast cancer.

40. A kit for detecting a cancer-associated marker protein present in a bodily fluid of a mammal, the kit comprising:

(a) mammalian autoantibodies directed against a cancer-associated marker protein from the same species as said autoantibodies; and

(b) means for detecting the formation of complexes between said autoantibodies and said cancer-associated marker protein.

41. A kit as claimed in claim 40 wherein said autoantibodies are human autoantibodies

42. A kit as claimed in claim 40 or 41 wherein said autoantibodies are human autoantibodies.

43. A kit as claimed in any one of claims 40 to 42 wherein said marker protein is a cancer-associated marker protein associated with lymphomas, leukaemias, breast cancers, colorectal cancers, lung cancers, pancreatic cancers, prostate cancers, cervical cancers, ovarian cancers, endometrial cancers or cancers of the skin.

44. A kit as claimed in claim 43 wherein said marker protein is a breast-cancer associated marker protein.

45. A kit as claimed in any one of claims 40 to 44 wherein said marker protein is a modified MUC1, BRCA1, BRCA2, p53, c-myc, c-erbβ2 or Ras protein.

46. A kit as claimed in claim 45 wherein said marker protein is a modified MUC1, BRCA1, BRCA2, c-myc, p53, c-erbβ2 or Ras protein associated with primary breast cancer.

47. A kit as claimed in claim 45 wherein said marker protein is a modified MUC1, BRCA1, BRCA2, p53, c-myc, c-erbβ2 or Ras protein associated with advanced breast cancer.

48. A method for detecting a cancer-associated marker protein present in a bodily fluid of a mammal substantially as described herein with reference to the accompanying examples.

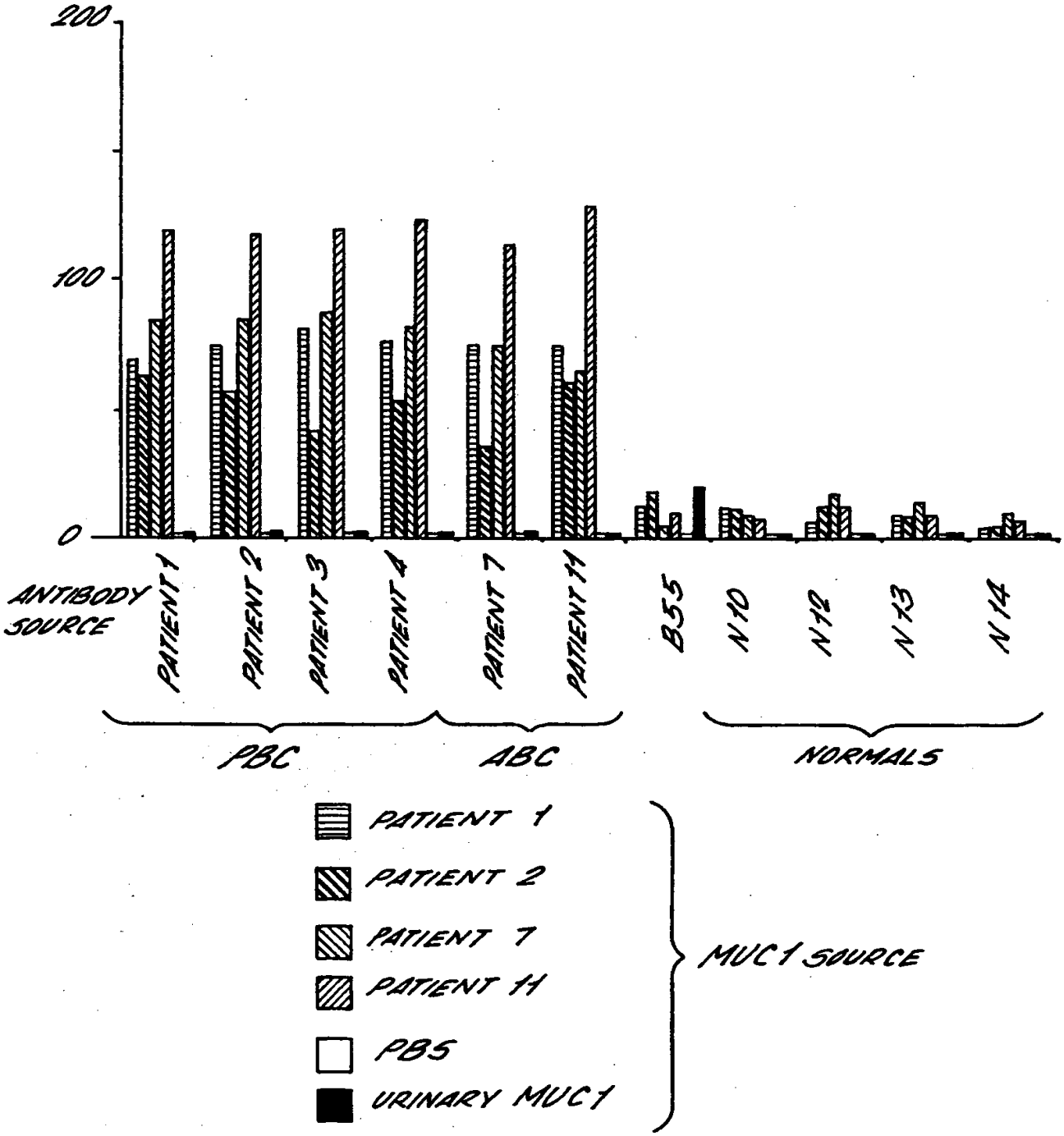
49. A kit for detecting a cancer-associated marker protein present in a bodily fluid of a mammal substantially as described herein with reference to the accompanying examples.

50. A diagnostic reagent substantially as described herein with reference to the accompanying examples.

51. An immortalized cell population capable of producing autoantibodies directed against one or more epitopes of a cancer-associated marker protein substantially as described herein with reference to the accompanying examples.

09/857739

FIG. 1.



THIS PAGE BLANK (USPTO)

FIG. 2.

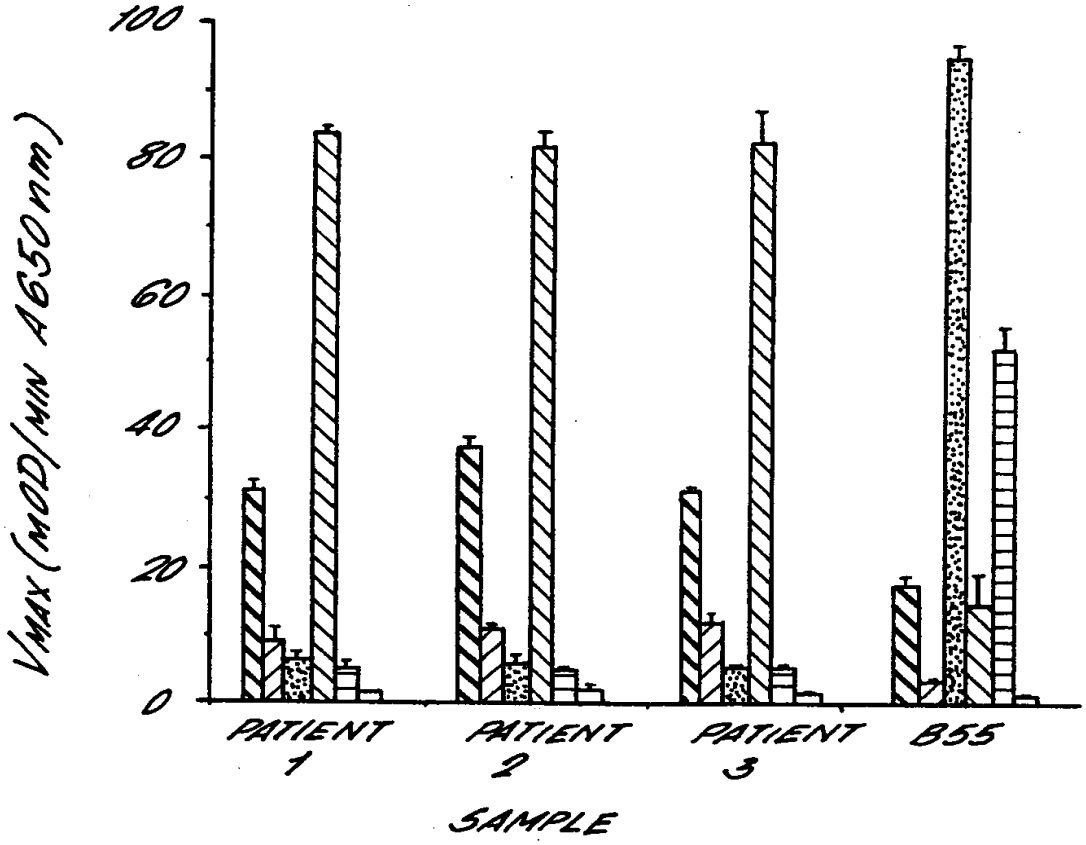
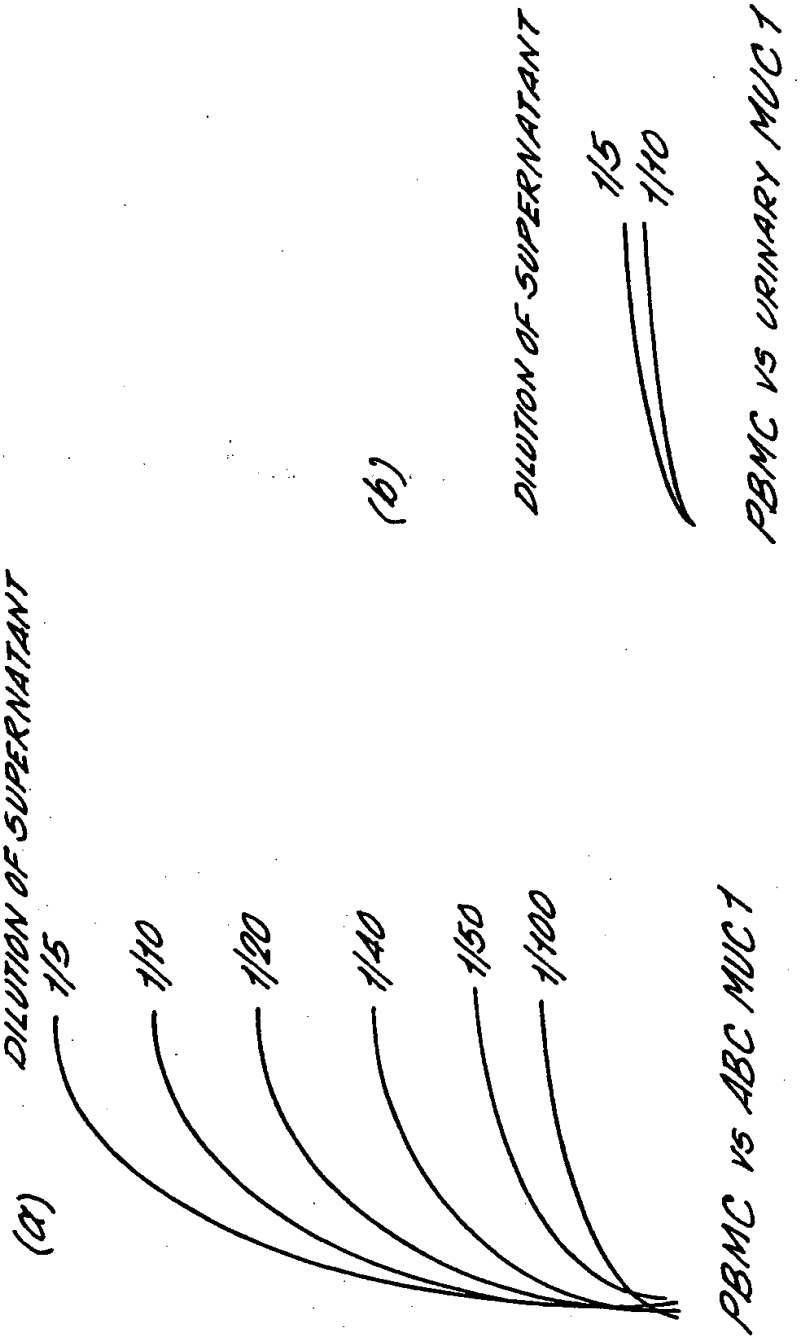

100
80
60
40
20
0
VMAX (MOD/MIN A650nm)
PATIENT 1
PATIENT 2
PATIENT 3
B55
SAMPLE
ABC MU1
CONTROL SERUM MUC1
IR75-1 MUC1
PBC MUC1
URINARY MUC1
PBS

FIG. 3.

SPR RESULTS DEPICTING THE RATE OF BINDING OF PBMC SUPERNATANT TO ABC AND URINARY MUC 1



THIS PAGE BLANK (USPTO)

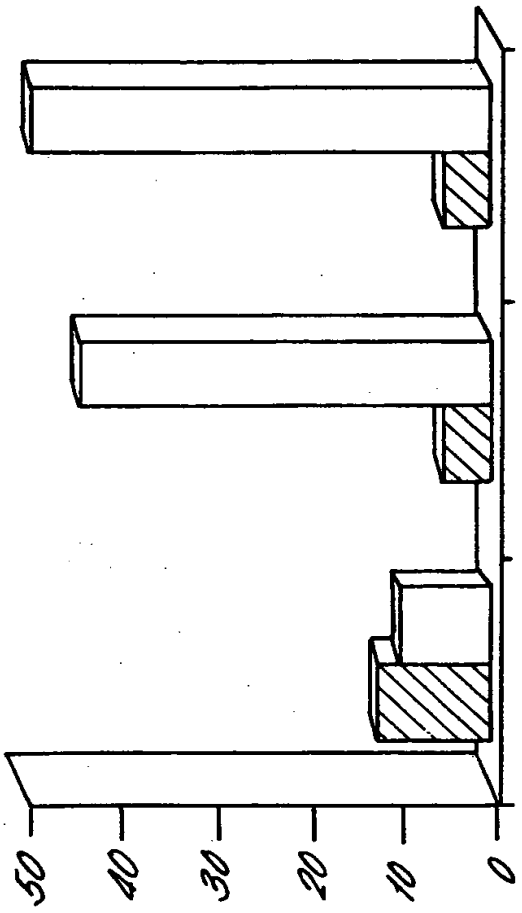
FIG. 4.

TAPPAHGVT*SAPDTRPAPGST*APPA

T* are O-glycosilated with N-acetyl-galactosamine

FIG 5.

SANDWICH ELISA TO DETECT MUC1
(NORMAL AND TUMOUR ASSOCIATED)



SANDWICHES OF :- 1 = C595/BIOTINYLATED C595
2 = PBC CULTURE SUP. } /BIOTINYLATED TAP2
3 = ABC CULTURE SUP. } PURIFIED ABC
SERUM ANTIBODIES

FIG. 6.

SANDWICH ELISA'S PERFORMED ON NORMAL AND CANCER SAMPLES

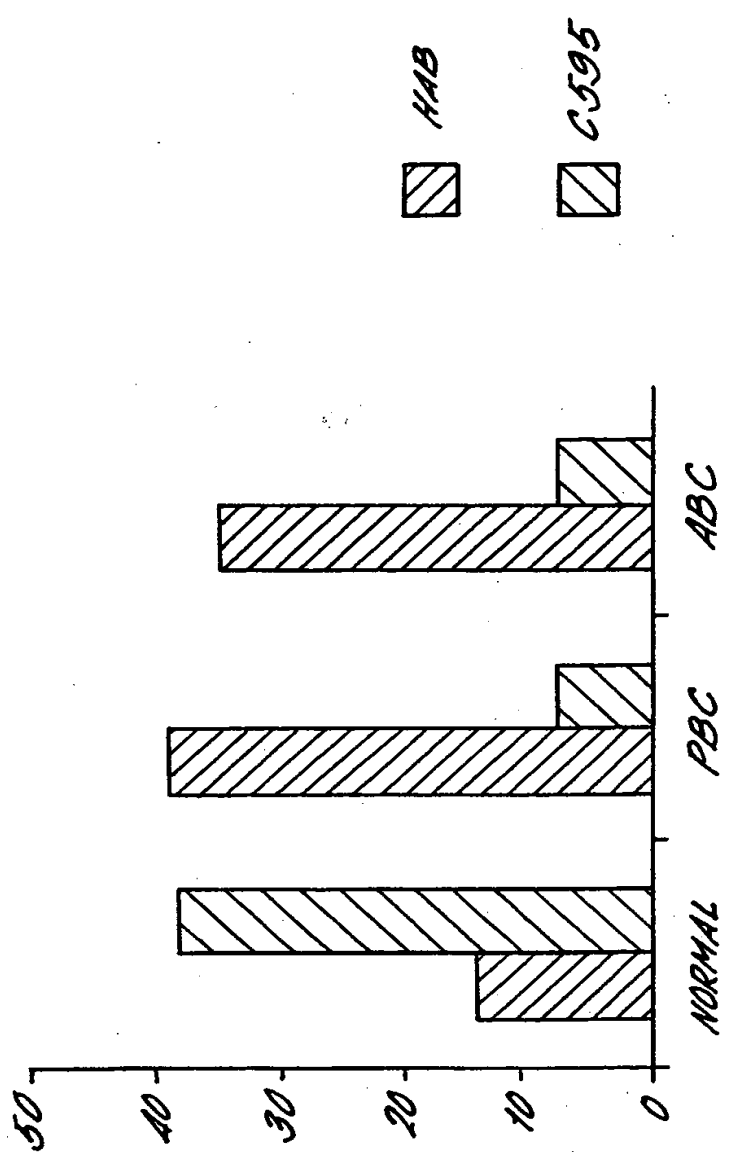


FIG. 7.

SANDWICH ELISA'S PERFORMED ON SEQUENTIAL SAMPLES FROM A PATIENT WITH METASTATIC BREAST CANCER.

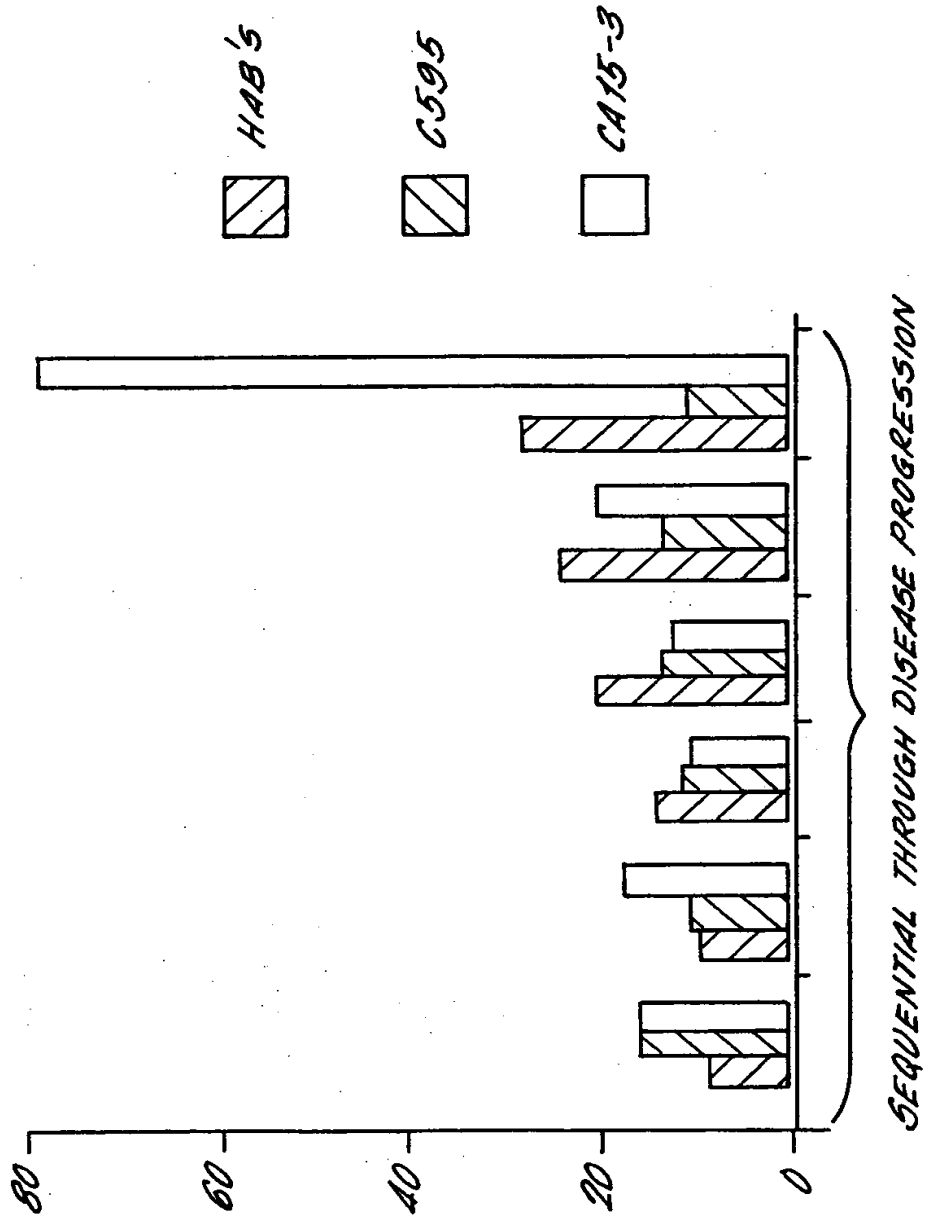
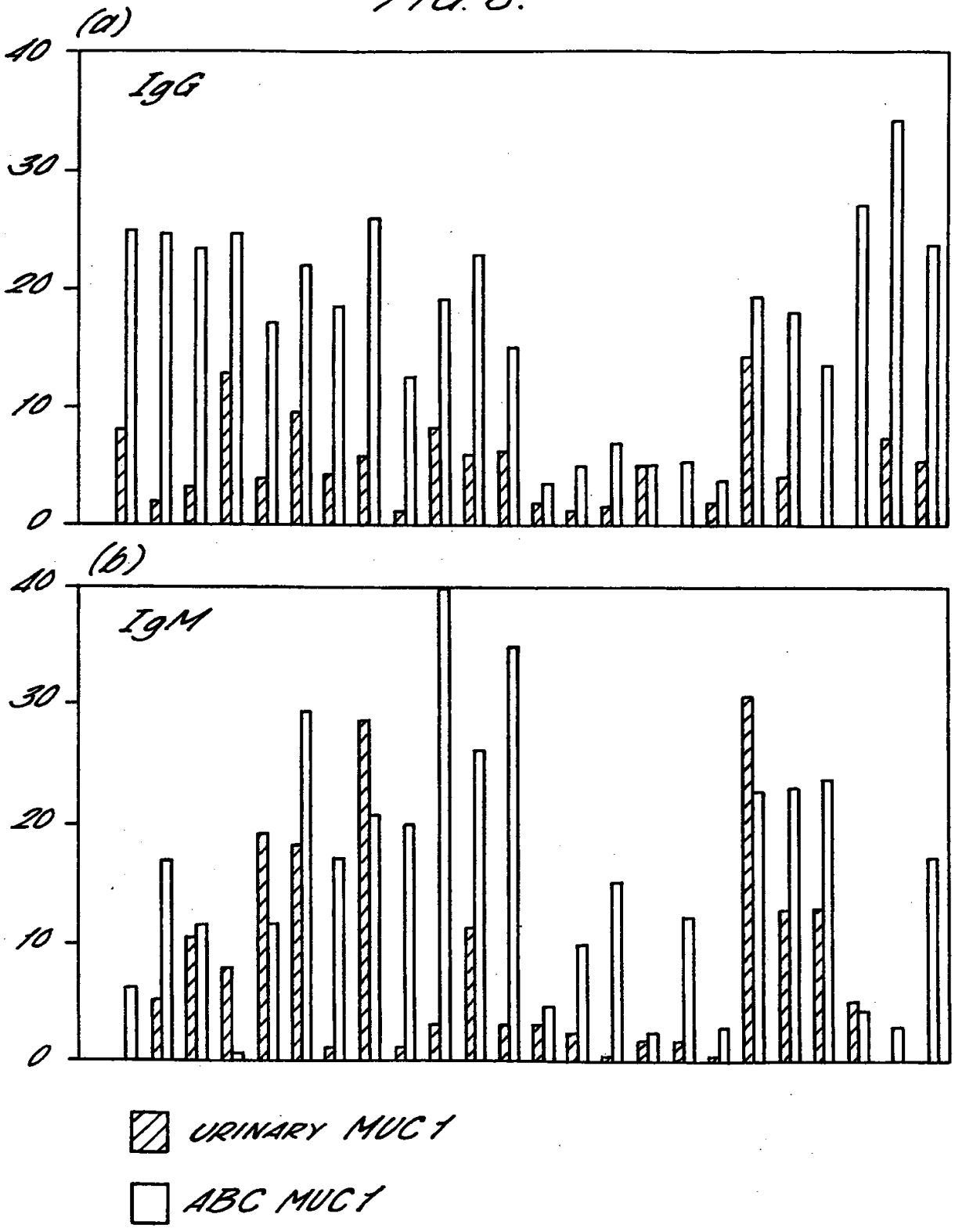


FIG. 8.



INTERNATIONAL SEARCH REPORT

Inte[redacted]onal Application No
P[redacted]B 99/04182

A. CLASSIFICATION F SUBJECT MATTER
IPC 7 G01N33/574 C07K14/47 G01N33/564 C12N5/06

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	E. PETRAKOU ET AL: "Preliminary studies on the binding of human autoantibodies to the MUC1 antigen." INTERNATIONAL JOURNAL OF ONCOLOGY, (1997) VOL. 11, NO. SUPPL., PP. 902. MEETING INFO.: 2ND WORLD CONGRESS ON ADVANCES IN ONCOLOGY ATHENS, GREECE OCTOBER 16-18, 1997, XP002115790 New York NY USA the whole document	1-51

-/—

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 March 2000	27/03/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Van Bohemen, C

2

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BIOLOGICAL ABSTRACTS, Philadelphia PA USA; abstact no. PREV199598348298, abstract XP002133115 & M. VOLKMANN ET AL.: "Anti-p53 autoantibodies as serological marker in different tumor-entities. " CLINICAL CHEMISTRY, vol. 41, no. S6 Part 2, 16 July 1995 (1995-07-16), pages S221-S222, WInston-Salem NC USA Meeting Info.: 47th Annual Meeting of the American Association for Clinical Chemistry, Inc. Anaheim, California, USA July 16-20, 1995	1-51
X	MEDLINE, Washington DC USA; abstract no. 93112867, abstract XP002133116 & R.E. LAFOND ET AL.: "Autoantibodies to c-myc protein: elevated levels in patients with African Burkitt's lymphoma and normal Ghanians" AUTOIMMUNITY, vol. 13, no. 3, 1992, pages 215-224, Boston MA USA	1-51
X,P	WO 99 58978 A (UNIVERSITY OF NOTTINGHAM) 18 November 1999 (1999-11-18) claims 1-71	1-51

2

INTERNATIONAL SEARCH REPORT

[redacted] on patent family members

Int[redacted]onal Application No
P[redacted]B 99/04182

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9958978 A	18-11-1999	NONE	

THIS PAGE BLANK (USPTO)